RESEARCH ARTICLE

Stability Test of Glycosaminoglycan and Achasin in Snail (*Achatina fullica*) Slime and Its Gel Formulation

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Received: 21th Dec, 19; Revised: 22th Jan, 20, Accepted: 16th Feb, 20; Available Online: 25th Mar, 2020

ABSTRACT

Snail slime (*Achatina fullica)* contains glycosaminoglycan, which can accelerate wound healing and achasin, which has antibacterial activity. The Objectives of the study to determine the stability of the protein in snail mucus in three different storage conditions. The snail slime stability test was carried out in two ways, namely the determination of protein content by the Lowry method and seeing the stability of the achasin and glycosaminoglycan proteins by the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS PAGE) method. In addition, the formulation of snail slime gel was made with a variety of gelling agents, namely Hydroxy Propyl Methyl Cellulose (HPMC) and Carbomer. Protein concentration showed a decrease in protein concentration, but the SDS-PAGE result showed that the decrease was not inactive protein (glycosaminoglycan and achasin). Further study should be conducted to evaluate stability in long time with different excipient and also the effectiveness of formulation in accelerate wound healing. According to the result, we are observed, the snail slime was successfully formulated into a gel with HMPC and carbopol as a gelling agent.

Keywords: Achasin, Carbopol, Glycosaminoglycan, HPMC, SDS-PAGE, Slime, Snail.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.1.2

How to cite this article: Agustina L, Shoviantari F, Aditya D. Stability Test of Glycosaminoglycan and Achasin in Snail (*Achatina fullica*) Slime and Its Gel Formulation. International Journal of Drug Delivery Technology. 2020; 10(1): 5-8.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Indonesia is a tropical country that has diverse flora and fauna potential; diverse natural potential needs to be explored, especially in the health sector. One traditional treatment that has been known for a long time is snail (*Achatina fulica)* slime to accelerate wound healing. Snails live almost all over the world; some live in a a land in humid places.[1] Snails live wild in various places, so it quickly spreads in the archipelago. Snails belong to the group of soft animals (Mollusca), which are included in the gastropod class. The body is soft and protected by a hard shell. This type of animal is spread in the sea, freshwater, and humid land.[2] Snail slime contains a variety of chemicals that have many benefits.

Snail slime contains achasin and glycosaminoglycan. Achasin functions as an antibacterial and anti-inflammatory factor.[3] This effect can accelerate the process in the inflammatory phase so that it will accelerate the proliferation phase in the wound healing process.[4] Glycosaminoglycans are composed of molecules from carbohydrates, dissolved globular proteins, uric acid, and oligo-elements (copper, zinc, calcium, and iron). Glycosaminoglycans from snails (*Achatina fulica*) are related to the heparin sulfate group, functioning to accelerate wound healing by helping the process of blood clotting and fibroblast cell proliferation.[5] Snail mucus with levels of 9% is known to be effective in healing wounds.[6] Besides that, snail mucus can provide a barrier to the growth of Staphylococcus aureus bacteria and Escherichia coli with a concentration of 8%, and able to kill the same bacteria a minimum level of 15% (MBC) and is effective in producing capable wound caps absorbs pus and accelerates wound healing.[7]

There are much research has been done on the protein content in snail mucus, and testing snail slime activity as an antibacterial or as a wound healer[8-10] but not yet carried out a protein stability test in snail mucus, whereas stability is one of the crucial problems in the development of pharmaceutical preparations. Stability is defined as the ability of a product to survive within its limits determined and throughout storage, nature, and characteristics are the same as those owned at the time the product was made. In general, the stability criteria include chemical, physical, microbiology, therapeutic and toxicology.[11] In this study, an initial stage will be conducted chemical stability testing, to determine the resistance of achasin and glycosaminoglycan proteins in gel preparations in three different storage conditions after storing for two weeks. Analysis of achasin and glycosaminoglycan protein stability using the Sodium method Dodecyl Sulphate-Polyacrylamide

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Gel Electrophoresis (SDS-PAGE). This method is used to characterize proteins from snail mucus based on molecular weight. SDS-PAGE got it. It is also used to observe the stability of the target protein that has been known to be heavy the molecules and measurements of protein levels by the Lowry method.

Wound healing with the traditional way of applying mucus to wounds is less hygienic and less attractive and therefore made in the form of gel preparations. Healing wounds by using snail mucus can be another alternative because of resources that are easily available, easy to use, good dispersion, do not clog pores, and have antibacterial activity.[12] The gel was chosen because it is a water-based gel or glycerin, which can help moisturize the skin so that it helps the wound healing process.[13] The gel also has a cooling effect when penetrating the skin, but it is more easily absorbed because it does not have a sticky effect such as preparations for creams or ointments because the gel is easily absorbed in the skin.[14] In this research, gels will be made with snail slime active ingredients using a variation of HPMC and Carbomer gelling agents, to find out which base is most suitable.

MATERIAL AND METHODS

Materials

Snail slime (*Achatina fullica*), HPMC, carbopol, aquadest, methyl paraben, propil paraben, gliserin, sodium dodecyl sulphate, acrylamide.

Methods

Snail slime collection

Snails were cleaned with aqua dest. Slime was collected by pressing and touching the snail body with sterile glass stick. Collected slime was then separated from its impurities by centrifugation at 10xg for 5 minutes.

Determination of snail slime protein Concentration

The stability of protein concentration in snail mucus is evaluated in the following manner; The samples were stored for two weeks under three different conditions namely 4°C, sun-protected room temperature, and sun-exposed room temperature. Protein concentration was determined by Lowry method. Protein concentration was calculated through a standard Bovine Serum Albumin (BSA) curve with a series of concentrations of 0, 200, 400, 600, 800, 1000 ppm.

SDS-PAGE analysis

The stability of protein achasin and glycosaminoglycan in snail slime was evaluated by SDS-PAGE analysis. Before running, a separating, stacking and marker gel was prepared. A sample was quantified using nanodrop and denaturated in boiling water. The electrode buffer contains 25 mM Tris HCl, 0.1% SDS, 190 Mm glycine.[4]

Gel Formulation

The following Table 1 is a formulation of snail slime gel with different gelling agents.

Gel stability test

The gel was evaluated on its appearance during 28 days of storage at room temperature protected from light, room temperature exposed to sunlight, and 4°C. The evaluation included organoleptic, homogeneity, spreadability, and pH.

pH measurement

pH measurement was conducted by using a digital pH meter. Before used, pH was calibrated using buffer pH 7.0 and 4.0. The electrode was dipping into the gel system. The pH measurement was conducted triplicate.[15]

Organoleptic

Homogeneity and physical appearance were observed through visual perception.

Spreadability

Two sides of glass slides were used in this assya. A hundred gram of gel was placed in the middle of the glass. The spreadability was then measure by calipers.

RESULT AND DISCUSSION

The protein concentration from *Achatina fullica*'s snail slime was quantified through the Lowry method. The quantification of protein aims to observe protein stability and prepared for SDS-PAGE analysis. A freshly collected snail slime showed a concentration of 438,96 µg. On the other hand, the concentration of snail slime that has been stored for two weeks at 4°C, room temperature, and room temperature exposed to sunlight were 401,6; 364.76; 331.87 µg. We observed the decrease in protein concentration as much as 9% in 4°C; 16.91% in room temperature, and 24.4% in room temperature and exposed to sunlight. The decrease in protein concentration indicated the instability, but it is required further observation

Table 1: Gel formulation of snail slime

	Formulation (%)					
Ingredients	*I*	*II*	*III*	*IV*	*V*	*VI*
Snail Slime	10	10	10	10	10	10
HPMC	2	3	4	-	-	-
Carbopol	-	-	-	3	4	5
Methylparaben	0.075	0.075	0.075	0.075	0.075	0.075
Propylparaben	0.025	0.025	0.025	0.025	0.025	0.025
Glycerin	10	10	10	10	10	10
Ethanol	1	1	1	1	1	1
Aquadest ad	100	100	100	100	100	100

Table 2: Protein concentration of snail slime in various storage

Snail Slime	*Protein Concentration (Mikrogram)*	*Percentage (%)*
Freshly extracted	438,96	100
Storage in 4° C	401,6	91
Storage in room temperature and protected from sunlight	364,76	83,09
Storage in room temperature and exposed to sunlight	331,87	75,60

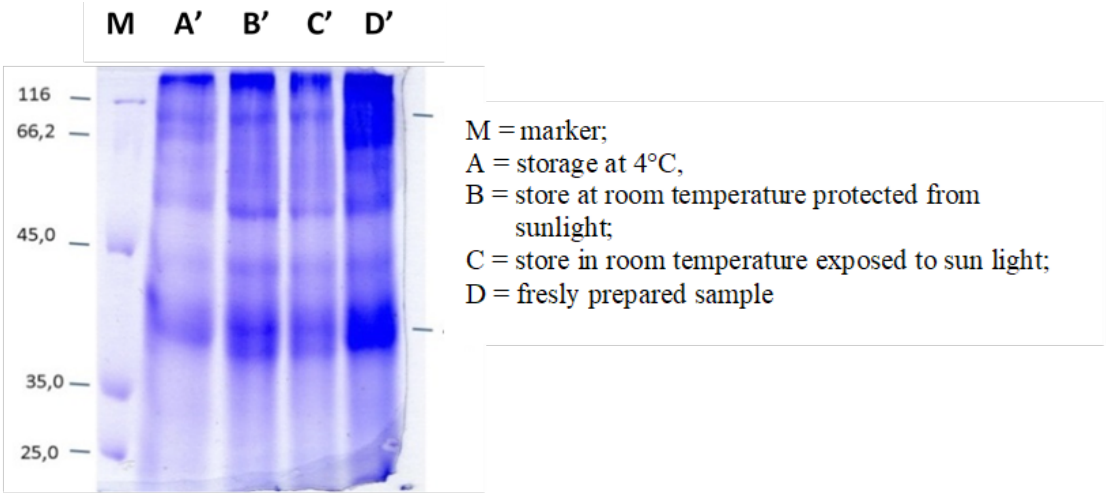
in SDS-PAGE to observed wether glycosaminoglycan, and achasin or other protein was decreased.

The SDS-PAGE is a method to analyze protein based on differences in molecular size. In our study, we used this technique to observed the presence of glycosaminoglycan and achasin, and also the consistency of specific band during storage. Glycosaminoglycan has a molecular weight of 40 kDa, while achasin was 71.3 kDa. From our result, we found a thick band with molecular size among 45-35 kDa, which we suggest as glycosaminoglycan, and 116-66 kDa as achasin. The suspected band was stable according to SDS-PAGE visualization, but the decrease of band thickness observed in another band.

The formula was optimized with two gelling agent, HPMC, and carbopol. In our study we used 2% (I), 3% (II) and 4% (III) concentration of HPMC; 3% (IV), 4% (V) and 5% (VI) of carbopol. Excipients used in our formulation, including methylparaben, propylparaben, glycerin, perfume, and aqua dest. The formula stability was observed during 28 days of storage. Evaluation of organoleptic, homogeneity, pH, and spreadability was carried out on 1, 3, 7, 14, and 28 days. The gel was placed in room temperature protected from sunlight, room temperature exposed to the sunlight, and 4°C.

The organoleptic evaluation showed that gel was transparent with a smooth texture and no change in gel color and aroma during storage. The homogeneity evaluation showed that the gel was homogenous. In the formula with carbopol as a gelling agent, we observed a few air bubbles entagled in the gel mass. Even though the stirring speed used in the formulation was reduced, the air bubble remains entangled. After the formula is allowed to stand for 3 days at 4°C, the air bubble disappears. From the organoleptic observation and homogeneity, all the formula was stable during 28 dyas of storage at different conditions.

The pH of formulation affects active compound stability and also influencing consumer preference. Prepared gel with



Picture 1: The result of SDS-PAGE.

Table 3: Organoleptic observation of gel containing snail slime (***A. fulica***)

Time (days)	*Formulation*																	
	I			*II*			*III*			*IV*			*V*			*VI*		
0	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
1	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
3	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
7	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
14	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
21	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H
28	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H	C	A	H

C = Clear ; A = Aromatic; H = Homogenoous

Table 4: pH assay of gel containing snail slime (***A. fulica***)

	Formulation					
Time (days)	*I*	*II*	*II*	*IV*	*V*	*VI*
1	4,72	4,75	4,76	4,02	4,25	5,67
3	4,72	4,72	4,74	3,91	4,21	5,60
7	4,72	4,72	4,73	3,87	4,13	5,58
14	4,70	4,69	4,70	3,79	3,92	5,56
21	4,70	4,67	4,69	3,76	3,90	5,54
28	4,70	4,65	4,69	3,70	3,87	5,54

Table 5: Spreadability of gel containing snail slime (***A. fulica***)

	Formulation (in cm)					
Time (days)	*I*	*II*	*III*	*IV*	*V*	*VI*
1	5	4,1	3,6	6	5,4	5,1
3	5	4,3	4,0	6,3	5,5	5,2
7	5,2	4,5	4,3	6,4	5,9	5,4
14	5,4	5	4,3	6,7	6	5,6
21	5,6	5,1	4,5	6,8	6,2	5,7
28	5,6	5,2	4,7	6,8	6,2	5,7

HPMC has a pH value range from 4.6–4.7, while gel with carbopol has pH value range from 4.0–5.5. pH for topical preparation should in ratio 4.5–6.5.[16] Our prepared gel is quite acid but still within the required specification of topical formulation. But, the irritation test should be conducted for further research to ensure safety. Moreover, we also observed a slightly decrease in pH value during storage. This decrease could indicate the instability in the formulation.

The value of spreadability indicated that the gel formulation was easily spread. According to Garge *et al.* (2002) the topical preparation should have spreadability range to 3–5 cm.[17] Our result showed that all gel meet the requirement, except for carbopol preparation (Formula I and II). Furthermore, we observe the increment in spreadability value during storage in all formulation.

CONCLUSION

According to the result, we are observed, the snail slime was successfully formulated into a gel with HMPC and carbopol as a gelling agent. All formulas showed good homogeneity and organoleptic. The formula is not stable during storage because we observed the decreased value in pH and increase in spreadability. Further observation with protein concentration showed a decrease in protein concentration, but the SDS-PAGE result showed that the decrease was not inactive protein (glycosaminoglycan and achasin). Further study should be conducted to evaluate stability in long time with a different excipient and also the effectiveness of formulation in accelerate wound healing.

ACKNOWLEDGMENT

Thank you to the Ministry of Research and Higher Education of the Republic of Indonesia for funding the budding lecturer research of the 2019 budget year.

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